motility quite different from that of any of the other preparations tested. The increase in motility was immediate, short-lived, and accompanied by a transient blood pressue depression of greater than 30 mm Hg. Blood pressure changes have not been seen following injection of villikinin(1). It can be concluded that bradykinin is not responsible for the villikinin-like action of plasma extracts.

The experiments shown were performed primarily using human materials. In addition to human plasma, extracts were made from bovine and canine plasma. Tests on these preparations similar to those outlined above gave identical results, as it was not possible to demonstrate any difference between human, bovine and canine plasma villikinin. Similarly it was not possible to demonstrate differences in human and canine urovilikinin. All of the villikinin preparations tested producted a reaction pattern which is similar to those shown in previous publications(1,9). While there may be differences in the amounts extracted from a particular source, *e.g.*, plasma, there can be little doubt that villikinin has been extracted from the plasma and that plasma villikinin is identical to mucosal and urovillikinin.

Summary. A series of experiments has been presented in which it was shown that crude extracts of human plasma contain a material that stimulates villous motility. It was not possible to distinguish a difference between the activities of crude mucosal, plasma, and urine preparations although it was suggested that there may be concentration differences. Refined preparations of villikinin showed a similarity to mucosal and urine extracts in activity as well as mobility. However, it was not possible to demonstrate antivillikinin in preparations other than those from intestinal mucosa. The activity of villikinin was different from that produced by histamine, 5-hydroxytryptamine and bradykinin. It is felt that the data presented clearly demonstrate the presence of villikinin in the plasma and that plasma villikinin is similar to mucosal and urovillikinin.

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Hepatic Microsomal Activities in Rats with Long and Short Sleeping Times after Hexobarbital: A Comparison.* (32072)

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It is well known that the primary site of drug-metabolizing activities in the mammal is in the microsomal fraction of the liver. The microsome-catalyzed oxidative reactions such as hydroxylation of aromatic rings; oxidation of aliphatic side chains to alcohols, ketones, or carboxylic groups; dealkylation from oxy-

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gen; and dealkylation from amino nitrogen all have common requirements for atmospheric oxygen and reduced triphosphopyridine nucleotide (NADPH)(1). Attempts to separate and purify the enzymes responsible for each of these oxidative reactions have not been successful.

Indirect evidence based on the differences (a) in the responses of various oxidative drug-metabolizing activities to microsome inducers(2,3) and to microsome inhibitors(4) and (b) in the relative activities of these oxidative pathways in various species(5), strains(5,6), and sexes(7,8), however, leaves little doubt that each oxidative reaction is catalyzed by a separate and distinct enzyme. In fact, experimental evidence for the presence of more than one aromatic hydroxylase(3,9), O-dealkylase(10), and N-dealkylase(11,12) has been reported.

It was therefore of interest to examine the various oxidative activities in rats grouped on the basis of their differences in response to one drug. The drug selected was hexobarbital; the difference in the duration of sleep was used to separate the rats into long- and short-sleepers. The microsomal activities were assayed with respect to 4 drugs representing 4 different drug-metabolism pathways. Five strains of rats were used in these comparative studies. Furthermore, the effect of phenobarbital pretreatment on rates of metabolism of acetanilide and aminopyrine by the liver preparations of long- and short-sleepers was examined in one rat strain.

Materials and methods. The Buffalo and Wistar rats were purchased from Microbiological Associates, Inc., Bethesda, Md. The other 3 strains were obtained from Simonsen Laboratories, Inc., Gilroy, Calif. Male rats (180-200 g) were used throughout. They were maintained on a stock diet and water *ad libitum* but were fasted for 15 hours before administration of hexobarbital.

To select long- and short-sleepers, 12 rats of each strain were injected intraperitoneally with 100 mg/kg of hexobarbital; the duration of loss of righting reflexes was recorded for each animal. Three each of the longest- and shortest-sleepers were chosen for subsequent enzyme studies. The set was not used unless the ratio of the average sleeping times between the long- and short-sleepers was approximately two. The experiment was repeated with one or two additional sets of 12 rats.

In the induction studies with long- and short-sleepers, 8 each of long- and short-sleepers were selected, as described above, out of a group of 30 Long-Evans rats. Starting on the next day, 4 from each group were injected intraperitoneally twice daily for 3 days with 38 mg/kg of phenobarbital. The other 4 from each group were kept as controls. The experiment was repeated with 2 additional sets of 30 rats.

Rats were killed either by cervical dislocation or by decapitation. They were sacrificed on the day of hexobarbital experiment. In the induction studies, the rats were sacrificed on the fourth day after the beginning of phenobarbital treatment. The livers were removed immediately and homogenized in an all-glass Potter homogenizer. Homogenates were routinely prepared using 3 ml of cold isotonic KCl (1.15%) for each gram of liver. A post-mitochondrial supernatant fraction containing the microsomes was prepared by centrifuging the homogenate in a Servall refrigerated centrifuge for 10 minutes at 10,000 $\times$ *g*.

To assay the drug-metabolizing activity, 1 ml of supernatant fraction (25-35 mg protein) was mixed with a solution containing tris buffer, pH 8.0 (200 μmoles), glucose-6-phosphate (25-40 μmoles), nicotinamide (15 μmoles), $MgCl_2$ (25 μmoles), NADP (0.25 μmole) NAD or NADH (5 μmoles), and various substrates in a final volume of 3.1 to 3.3 ml. Using a Dubnoff metabolic shaker incubation was carried out for 30 minutes at 37°C in air.

The metabolic pathways, the amounts of substrate per beaker, and the assay methods used were:

(a) side-chain oxidation of hexobarbital, 2 μmoles, optical density measurement at 245 mμ of the unmetabolized hexobarbital. The extraction procedure used was that of Brodie *et al*(13) except that heptane containing 1.5% isoamyl alcohol was used.

(b) O-demethylation of o-nitroanisole, 7 μmoles. The amount of o-nitrophenol formed

TABLE I. *In vitro* Rates of Drug Metabolism of Short and Long Sleepers in Various Strains of Rats.

Strain of rats	No. of rats	Hexobarbital sleeping time (min)	μmoles of substrate metabolized or product formed per g of protein: Hexobarbital	o-Nitroanisole	Aminopyrine	Acetanilide
Long-Evans	9	50 ± 8	31.6 ± 5.5	9.1 ± 1.5	2.2 ± .1	8.5 ± 1.3
	9	26 ± 5	41.5 ± 3.2	11.1 ± 1.6	3.6 ±1.1	10.0 ± 1.2
			p <.001	p <.025	p <.01	p <.025
Sprague-Dawley	9	55 ± 8	30.0 ± 3.0	6.6 ± .7	1.1 ± .3	8.8 ± 1.9
	9	29 ± 3	42.9 ± 5.4	8.4 ± 1.3	2.1 ± .7	12.4 ± 2.3
			p <.001	p <.005	p <.005	p <.005
Buffalo (inbred)	9	65 ±14	24.9 ± 9.0	7.5 ± 1.1	1.1 ± .6	5.8 ± 1.8
	9	32 ± 6	36.4 ± 5.4	8.9 ± .7	2.0 ± .7	7.4 ± 1.1
			p <.005	p <.01	p <.01	p <.05
Fischer (inbred)	9	43 ±11	41.4 ± 3.1	10.4 ± 1.9	2.0 ± .2	12.8 ± 1.2
	9	19 ± 9	47.9 ± 5.4	12.2 ± 2.0	2.9 ± .7	16.6 ± 3.1
			p <.01	p >.05*	p <.005	p <.005
Wistar	6	56 ± 6	35.2 ± 2.5	8.2 ± .2	1.8 ± .3	11.4 ± 2.7
	6	30 ± 5	44.6 ± 2.6	10.2 ± .9	3.0 ± .7	15.4 ± 3.0
			p <.001	p <.001	p <.005	p <.05

* Not significantly different from each other. All values are the means ± S.D.

was measured spectrophotometrically(14).

(c) N-demethylation of aminopyrine, 10 μmoles. The amount of 4-aminoantipyrine formed was measured by the method of LaDu(15).

(d) aromatic ring hydroxylation of acetanilide, 10 μmoles. The amount of p-hydroxyacetanilide formed was measured colorimetrically by the method of Ninomiya(16).

Protein concentration was determined by the method of Lowry *et al*(17). The enzymic activities were expressed as μmoles of substrate metabolized or product formed per gram of protein in 30 minutes. Comparisons of the mean enzymic activities between long- and short-sleepers and between control and phenobarbital treated rats were made by student's "t" test.

Results. The duration of sleep and the drug-metabolizing activity of long- and short-sleepers in 5 strains of rats are shown in Table I. As expected, the activity of the liver preparations in the metabolism of hexobarbital was less in long-sleepers than in short-sleepers. The metabolic rates of 3 other drugs representing different metabolic pathways were also consistently less with the liver preparations from long-sleepers than with those from short-sleepers. This trend was observed in all 5 rat strains examined except in the metabolism of o-nitroanisole by the Fischer rats. It may therefore be generalized that a slow metabolizer of one drug metabolizes other drugs slower than does a fast metabolizer.

No marked strain differences were observed in the metabolic rates of drugs. The livers of Wistar and Fischer rats showed the highest and Buffalo rats the lowest drug metabolizing activities among the five strains studied.

Data presented in Table II show that after phenobarbital pretreatment, there is no longer any difference in acetanilide and aminopyrine metabolism between the long- and short-sleepers.

Discussion. These experiments indicate that rats that are slow in metabolizing a drug through one metabolic pathway are also slow in metabolizing other drugs through different metabolic pathways. The present finding is confined to those drugs detoxified oxidatively by the liver microsomes. This implies that an individual who shows a long duration of action against one drug might also show a long duration of action against other drugs. This observation holds if drug metabolism exclusive of metabolism to its active metabolite is the major consideration in determining drug response; however this is not always the case. For instance, Elbert *et al*(18) could

TABLE II. Induction Experiments with Long and Short Sleepers.

Groups	No. of rats	Hexobarbital sleeping time (min)	μmoles of product formed per g of liver protein			
			Acetanilide		Aminopyrine	
			A	B	A	B
Long sleepers	24	48 ± 9	8.5 ± 1.8	16.7 ± 4.0	1.7 ± .6	7.3 ± 1.7
Short sleepers	24	22 ± 4	10.8 ± 3.0	17.2 ± 4.8	2.6 ± 1.1	7.9 ± 1.9
			$p < .05$	N.S.	$p = .025$	N.S.

Values under A and B refer to enzymic activities of untreated and phenobarbital-treated rats, respectively. Long-Evans male rats weighing approximately 200 g were used in this experiment. All values are the means ± S.D.

find no qualitative or quantative differences in the metabolic patterns of barbital in barbital-tolerant and -nontolerant rats.

The present finding was somewhat unexpected in view of the fact that various drug-metabolic pathways appear to be catalyzed by different enzymes; the indirect evidence for this view was cited in the introductory remarks. One plausible explanation for this consistent slowness or fastness in the metabolism of various drugs in a given group is that the rate of drug metabolism is limited by the enzyme system common to all oxidative pathways; namely, the enzyme concerned with the trapping or fixation of the atmospheric oxygen in the biologically usable form. It may be that in the uninduced state, the slow metabolizer has less of this common enzyme than the fast metabolizer has. That this enzyme system can be limiting under certain conditions was shown by Rubin *et al* (19), who demonstrated that hexobarbital, acetanilide, chlorpromazine, and other drugs competitively inhibited the N-dealkylation of ethylmorphine by rat hepatic microsomes. The fact that slow metabolizers can attain elevated activities similar to those in the fast metabolizers after phenobarbital induction indicates that the potential ability to metabolize drugs faster is present to the same extent in the two groups within a strain, but that under ordinary physiological conditions the drug-metabolizing capability is more subdued in the slow group than in the fast group.

The present findings also show that the variation in the basal metabolic rates in drug metabolism among the 5 strains of rats examined is not as great as that in different rabbit strains. Cram *et al*(6) found as much as 10-fold difference in the metabolism of hexobarbital and aminopyrine among different strains of rabbits. Approximately a 2-fold difference was reported in the sleeping time between 2 strains of mice(20) and rats(5). A difference of this magnitude in the sleeping time was observed within a given strain of rats in the present report.

Summary. Rats were separated into 2 groups—long-sleepers and short-sleepers—on the basis of their response to a dose of hexobarbital. The drug-metabolizing activities of the hepatic postmitochondrial supernatant fraction from long-sleepers were less than those from short-sleepers with respect to hexobarbital, acetanilide, aminopyrine, and o-nitroanisole, each of which represents a different drug-metabolism pathway. This trend was observed in all 5 strains of rats examined. After phenobarbital pretreatment, this difference between the two groups was no longer observed in one strain studied. The strain differences in drug-metabolism activities were not as great in the rat as those reported in various rabbit strains.

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Carbon Dioxide Seizures in Immature Rats.* (32073)

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It is well known that both exposure to and withdrawal from high concentrations of CO_2 can produce seizures in experimental animals and man(1,2). Although the exact mechanisms responsible for these seizures have not been elucidated, Woodbury and his associates have presented evidence that changes in central nervous system excitability caused by CO_2 are related to rapid changes in brain intracellular bicarbonate levels which, in turn, result in changes in brain cell pH (3,4).

In a recent paper(5), it was reported that immature rats, as contrasted with adult animals, have very high cellular bicarbonate levels in brain. Thus, in view of the intracellular acid-base data of Woodbury and coworkers cited above, very young rats might be expected to have different seizure responses to CO_2 challenges than do adult animals. This report is a summary of experiments in which this possibility was studied in detail by comparing the responses of immature and adult rats to various high CO_2 atmospheres.

Methods. General. Entire litters of Sprague-Dawley baby rats along with their mothers were obtained from a local supplier. All immature animals less than 3 weeks old remained with their mothers in their own cages until used; hence, variations in body temperature and fluid balance were minimized. Selection of young animals for tests was on the basis of age only; no attempt was made to group animals according to sex, weight, etc. Animals classified as adults in the results reported below were male Sprague-Dawley rats of 200-300 g body weight.

CO_2 Seizures. Animals of different ages were placed in a plastic chamber filled with various high concentrations of CO_2 and 20% oxygen. Usually, a total of 6 to 10 animals was observed at one time. Care was taken to have a wide representation of ages in each experimental group. Because of reported tolerance to CO_2(6) and adverse effects of the gas on growth(7), each animal was exposed to CO_2 only once. If an animal exhibited either jaw or forelimb clonus, or both, while being exposed to CO_2 in the plastic box, this animal was said to have had an in-chamber or exposure seizure.

After remaining in the chamber for approximately 8 minutes, all animals were taken from the plastic box and allowed to breathe room air. In susceptible animals, so-called withdrawal seizures produced by this maneuver were manifested as very rapid jaw and forelimb clonus, marked salivation, piloerection, and a rearing-up movement.

Additional details of the apparatus and

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